THE ACTION OF ARYL-STIBINIC ACIDS IN EXPERIMENTAL TRYPANOSOMIASIS

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From the Runcorn Research Laboratories of the Liverpool School of Tropical Medicine

(Received for publication 3 April, 1909)

Plimmer's and Thomson's important discovery of the trypanocidal action of Antimony, an element chemically closely allied to Arsenic, was the starting point for extensive investigations on the value of different Antimony compounds in the treatment of Trypanosomiasis. In their first experiments[1] they used Potassium antimonyl tartrate, and observed that this drug destroyed the trypanosomes in the peripheral circulation more rapidly than Atoxyl. The injections caused neither pain nor inflammatory changes of the tissue. In their experiments, out of twenty-five rats only four showed recurrences; nine lived for over two hundred days, and nine considerably over one hundred days; the remaining three died without any symptoms of Trypanosomiasis, and in none were trypanosomes found after death.[2]

Mesnil and Brimont[3] were able to confirm Plimmer's and Thomson's observations concerning the powerful trypanocidal action of Potassium antimonyl tartrate. Their experiments on several strains of pathogenic trypanosomes, however, proved that after one injection the parasites usually reappeared, sometimes after a very short time. In Ngana infected rats, as many as nine relapses were observed, and the drug had always the same transitory effect; most of the animals died finally either with or without parasites after discontinuation of the treatment. Their results in animals infected with *T. evansi*, were more satisfactory; and it is specially noteworthy

that an Atoxyl-resistant Surra strain reacted to Antimony in the same way as a normal strain. A preventative action was only noticed if the drug and the parasites were injected simultaneously in two different distant places.

In our hands,[4] Sodium antimonyl tartrate did not give such promising results in the treatment of rats, a fact which might be due to the use of an especially virulent strain of *T. equiperdum*. One horse injected with a strain of cattle trypanosomes brought back from the Congo, and one donkey infected with an Atoxyl-resistant strain of Ngana, were treated with Sodium antimonyl tartrate in fairly large doses. In both cases the drug caused the prompt disappearance of the parasites; the interval between the relapses, however, became shorter and shorter after each injection, and finally both animals succumbed to the disease.

Manson[5] was the first to administer Sodium antimonyl tartrate to a case of Sleeping Sickness. As Atoxyl given in large doses did not seem to have any effect on the trypanosomes, a treatment of Antimony in small doses was begun. It caused the disappearance of the parasites, but eighteen days afterwards parasites were again seen in the peripheral circulation. As the injection had caused intense irritation and pain, two grains of Antimony were given by the mouth; this was followed by nausea, and seemed to increase the mental depression of the patient. Antimony treatment was discontinued, and Atoxyl again given.

For further experiments Mesnil and Brimont[6] used mice infected with different laboratory strains of pathogenic trypanosomes. According to their results they were able to separate the strains into two groups. The parasites of the first group disappeared after a single injection: Surra and Dourine belonging to this group. In the second group, to which all other strains belong, the parasites disappeared after the injection of Tartar emetic, but only to reappear within a few days. The negative phases after each injection became shorter and shorter, and the animals finally succumbed to the disease.

In the discussion, Laveran[7 a, b] states that in his hands, Tartar emetic did not prove very satisfactory in guinea-pigs, as after the rapid disappearance of the parasites following the first injection, relapses occurred very frequently. Sulphide of Antimony, in his experience, is much less active than Sulphide of Arsenic (orpiment).

Uhlenhuth and Woithe[8] repeated the experiments with Sodium antimonyl tartrate on twenty-seven rats infected with *T. equiperdum*, but their results were as discouraging as those with Arsenious acid. Even repeated injections of 0·003—0·005 gramme of the Sodium salt and 0·002—0·003 gramme of the Potassium salt were unable to effect even temporary disappearance of the parasites from the blood.

Broden and Rodhain[9] used soluble as well as insoluble compounds of Antimony in Sleeping Sickness treatment. The hypodermic injections caused great irritation and pain, and were followed, even when given intramuscularly, by large swellings; this reaction persisting for some days, and only disappearing after one week. Therefore, the drug was administered intravenously. A dose of 0·07 gramme was sufficient in some cases to cause the parasites to disappear, but they very soon reappeared in the peripheral circulation. These observers recommend a dose of 0·1 gramme of Tartar emetic. This dose given intravenously did not usually cause any severe symptoms; it was followed sometimes by profuse perspiration and vomiting. After repeated injections the patients usually lost their appetite and complained of general malaise. On interruption of the treatment these symptoms passed off. They were able to confirm the rapidity of the destructive action of Antimony on the parasites, but remark that, ' Ces constatations doivent nous imposer une extrème reserve dans l'appréciation de la valeur de l'antimonie dans le traitement de la trypanosomiasis humaine et éxigeront une experimentation longue et patiente.'

They, however, place, for the present at least, the soluble Antimony compounds on the same level as Atoxyl.

Broden and Rodhain, as well as Martin and Darré,[10] combined the Atoxyl treatment of Sleeping Sickness patients with injections of soluble Antimony compounds with very encouraging results.

Good results of Antimony treatment in experimental Syphilis are recorded by P. Salmon,[11] and Broden and Rodhain[9] were able to confirm the beneficial effect of Antimony in human Syphilis.

Plimmer's and Thomson's observations on the rapid action of Potassium antimonyl tartrate on trypanosomes in the blood of infected animals seemed to justify an attempt to prepare an organic Antimony compound analagous to Atoxyl. Moreover, a comparison of the effects of injections of Sodium arsenate with those of Atoxyl made it

probable that injections of the Sodium salt of Amino-phenyl-stibinic acid would be far less irritating and permit the introduction of a larger amount of Antimony into the organism without toxic symptoms.

After many unsuccessful attempts, we succeeded in preparing the *p.m.* and *o.* amino-phenyl-stibinic acids. The action of the *p.* and *m.* compounds has been studied on experimental animals infected with various laboratory strains of pathogenic trypanosomes; the *o.* compound was, after a few tentative experiments, given up as impracticable.

I. EXPERIMENTS WITH *m.* AMINO-PHENYL-STIBINIC ACID

m. amino-phenyl-stibinic acid was used in the form of its acetylated derivative. This latter proved itself in the first experiments the less toxic, and produced no appreciable irritation at the site of inoculation, whereas the *m.* compound caused marked swellings and abscesses.

A. Rats.

Medium-sized rats of the weight of 180-220 grammes were used for the following experiments. Untreated animals succumbed to an infection of *T. brucei* in 4-6 days on the average. Treatment was usually started when numerous parasites were present in the peripheral blood. In a few experiments the compound was injected only at a late stage, some hours before death; but then the animal always died from the infection. The toxic dose was found to be 0·75 c.c. of a 5% solution; an injection of 0·5 c.c. of the same solution corresponding to 0·025 gramme of the drug was usually well borne. No abscesses were noticed at the site of injection. After the administration of this dose the parasites disappeared from the blood in 12-16 hours. Smaller doses were only able to effect a disappearance of the parasites from the blood, when repeated. The parasites, however, often reappeared after a comparatively short time; a further injection of the drug again resulting in the disappearance of the parasites.

Table I shows the details of the result of treatment on thirty-three rats: only one rat is still living after 136 days.

Rat No.	Disease	Date of inoculation	Treatment at the end of —	c.c. of 2% solution	Subsequent	Remarks
282 A.	*T. brucei*	Oct. 20	2, 3, 8, 23 days	2 c.c.	—	Lived 93 days. Abscesses of lung.
B.	,,	,,	2, 3, 8 days	1·5 c.c.	—	,, 10 ,, Kidney lesions; spleen small.
288 A.	,,	Oct. 26	2 days	0·75 c.c.	—	,, 5 ,, Antimony poisoning.
B.	,,	,,	,,	0·75 c.c.	—	,, 5 ,, ,, ,,
293 A.	,,	Oct. 30	2, 13 days	1 c.c.	—	,, 19 ,, No signs of Trypanosomiasis.
B.	,,	,,	,,	1 c.c.	—	,, 59 ,, ,, ,, ,,
254 A.	,,	Oct. 17	3 days	1·25 c.c.	—	,, 4 ,, Late stage of infection. Tryps. still present at death.
B.	,,	,,	,,	1·25 c.c.	—	,, 4 ,, ,, ,, ,,
C.	,,	,,	,,	1·25 c.c.	—	,, 4 ,, ,, ,, ,,
D.	,,	,,	,,	1·25 c.c.	—	,, 4 ,, ,, ,, ,,
255 A.	,,	Oct. 17	3 days	1 c.c.	—	,, 4 ,, Late stage of infection. Tryps. present at death.
B.	,,	,,	,,	1 c.c.	—	,, 4 ,, ,, ,, ,,
C.	,,	,,	,,	1 c.c.	—	,, 4 ,, ,, ,, ,,
D.	,,	,,	,,	1 c.c.	—	,, 4 ,, ,, ,, ,,
256 A.	,,	Oct. 22	3 days	—	—	Died before injection.
B.	,,	,,	,,	0·6 c.c.	—	Lived 4 days. Late stage of infection. Tryps. present at death.
259 A.	,,	Oct. 28	2, 14. 25 days	1·25 c.c.	25, 30 days	36 days. Tryps. present at death.
B.	,,	,,	2 days	0·5 c.c.	—	3 days.
262 A.	,,	Nov. 11	3 days	0·3 c.c.	—	3 days. Treatment started 2 hrs. before death. Tryps. present at death: slightly decreased in number.
B.	,,	,,	,,	0·3 c.c.	—	,, ,, ,, ,,
C.	,,	,,	,,	0·3 c.c.	—	,, ,, ,, ,,
D.	,,	,,	,,	0·3 c.c.	—	,, ,, ,, ,,
263 A.	,,	Nov. 12	2, 3, 9, 11, 12, 18 days	0·5 c.c.	9, 18	25 days. Blood swarming with Tryps. at death.
B.	,,	,,	,, ,,	0·5 c.c.	—	4 days. Tryps. absent at death.
264 A.	,,	Nov. 12	2, 3, 9, 11, 18 days	1·75 c.c.	18	22 days. Blood swarming with Tryps. at death.
B.	,,	,,	,, ,,	1·75 c.c.	—	Animal living after 136 days.
183	*T. gambiense*	June 29	38, 136 days	1·0 c.c.	85, 135	140 days. Tryps. absent at death.
257 A.	,,	Oct. 28	16, 17, 26, 27 days	1·05 c.c.	47	62 days. Tryps. present at death.
B.	,,	,,	16, 17 days	0·5 c.c.	—	20 days. Tryps. absent at death; spleen enlarged, kidneys normal.
C.	,,	,,	16, 17 days	0·5 c.c.	22	23 days. Tryps. present at death.
D.	,,	,,	16, 17 days	0·5 c.c.	—	18 days. Tryps. absent at death.
E.	,,	,,	16, 17, 26, 27 days	1·05 c.c.	—	32 days. ,, ,,
F.	,,	,,	16, 17, 26, 27 days	1·05 c.c.	—	28 days. ,, ,,

B. Dogs.

Two dogs were used for the experiments (see Table II). In experiment 245 the dog was treated with Sodium-amino-phenyl-stibinic acid; in experiment 244 with the acetylated derivative of the same compound. In the former experiment severe abscesses resulted from the injections, and after a very short time the animal succumbed to the toxic effects of the drug. The post-mortem examination revealed a haemorrhagic nephritis.

The subcutaneous injections of the acetylated compound, on the other hand, did not cause any irritation. Only frequently repeated large doses effected a disappearance of the parasites from the blood. Very soon, however, trypanosomes reappeared again. The animal succumbed to a severe toxic haemorrhagic nephritis due to the Antimony.

C. Rabbits.

Six rabbits were inoculated with *T. brucei*, and after the disease had become well established treatment was begun. But even prolonged administration of fairly large doses—0·1 gramme per injection—was not able to cope with the disease, and all the animals succumbed to the infection. Although parasites were very rarely seen in the peripheral blood, the well-known symptoms of a Ngana infection were more or less pronounced during the whole course of the treatment.

D. Guinea-pigs.

Treatment was only attempted in the case of three guinea-pigs, as it was soon apparent that these animals did not bear well, effective doses of Antimony. It was found that if this drug was administered in sufficiently large doses to destroy the parasites, the animal died from severe kidney lesions; small doses, on the other hand, did not, even if administered repeatedly, have a noticeable effect on the parasites in the blood.

It was noticed that on standing the *m.* amino-phenyl-stibinic acid lost its action on trypanosomes, and caused on injection serious toxic symptoms. This fact was due to a decomposition of the compound into Aniline and Antimonic acid.

TABLE II.

Exp. No.	Disease	Weight	Date of inoculation	Treatment at the end of—	Amount injected	Relapse after	Remarks
244	*T. brucei*	9 k. 600 grs.	Aug. 7	4, 5, 6, 7, 13, 15, 23, 50, 53, 55 days	6·4 gm.	11, 21, 31 days	Lived 60 days. From 31st to 54th day Tryps. present; absent at death. Cause of death—Haemorrhagic Nephritis.
245	,,	8 k. 700 grs.	Aug. 7	4, 5, 6, 7 days	1·1 gm.	—	Lived 14 days. Tryps. absent at death. Cause of death—Haemorrhagic Nephritis.

II. *p.* AMINO-PHENYL-STIBINIC ACID

After the somewhat discouraging results obtained with the use of *m.* Amino-phenyl-stibinic acid, experiments were undertaken with a view to ascertaining whether the *p.* compound was superior in its action to the *m.* compound.*

A. Rats.

Rats infected with *T. brucei*, *T. evansi*, and *T. gambiense* were used in the following experiments:—

The strain of *T. gambiense*, with which the experiments were carried out, was an especially virulent strain. It was recovered from a monkey at the time of its last relapse, a few days before death. Rats succumbed to the infection, on an average, 3-4 days after inoculation.

As a routine method of treatment, after some preliminary experiments, the following procedure was decided upon:—1st day, one injection of 0·5 c.c. of 5% solution, followed on the 3rd day by 0·25 c.c. of 5% solution. The injections were repeated after a varying interval, as seen in Table III, pp. 374-375.

This mode of treatment was found to be superior to injections of 0·5 c.c. of 5% solution on two subsequent days. Only a small percentage of the rats succumbed to the poisoning effects of Antimony, which took the form of a severe diarrhoea. At the post-mortem the mucous membrane of the intestine was markedly oedematous and inflamed; the kidneys showed all the signs of an acute inflammation.

The effect of the injection of the compound on the trypanosomes was very marked. The parasites disappeared usually after 12-16 hours. If treatment was discontinued the parasites reappeared in a comparatively short time, and a further injection again caused their prompt disappearance. Occasionally, after repeated injections the interval between relapses became shorter and shorter, until, finally, an injection of the drug had no influence at all on the parasites, and the animal died from trypanosomiasis. Table IV gives the details of these experiments. We were able to confirm Mesnil's and Brimont's[12]

* It is a very interesting observation of Ehrlich's that the *m.* Amino-phenyl-arsenic acid is markedly inferior in its therapeutic value to the *p.* Amino-phenyl-arsenic acid (Atoxyl). (Private communication.)

observation that this strain is not resistant to the drug in the same sense as Atoxyl resistant strains. If subinoculated into new rats, an injection of the Antimony compound caused a prompt disappearance of these parasites from the blood.

B. Dogs.

Experiments with dogs infected with *T. evansi* and *T. brucei* showed that these animals are very susceptible indeed to the toxic effect of the *p.* Antimony compound. If small doses were administered, the parasites did not disappear from the blood. If the doses were increased, the animal died in a very short time with severe kidney lesions. At the post-mortem, the kidneys were swollen and congested; subcapsular and cortical haemorrhages were noticed. The urine was of a slight reddish colour, containing red blood corpuscles, casts, and large quantities of albumen. Table V, p. 377, gives the details of these experiments.

Preliminary experiments on guinea-pigs proved that these animals reacted to the *p.* compound in the same way as to the *m.* compound.

C. Monkeys.

Monkeys infected with *T. gambiense* were used for the following experiments. Treatment was usually begun when the infection was well established, and the animals presented undoubted signs of illness. Two monkeys were treated at a late stage of the infection, two at an earlier stage with the *p.* compound. Two others were treated with a combination of *p.* amino-phenyl-stibinic acid and Atoxyl.

Experiment No. XV.—*Cercopithecus callitrichus*, weight 2 k. 920 gm. Treatment was begun seventy-two days after infection. Numerous parasites were then found in the blood. The animal was injected with 0·2 gm. of the *p.* compound. The parasites had disappeared by the next morning. Symptoms of Antimony poisoning had, however, set in: the monkey was vomiting white, slimy masses, and was suffering from severe diarrhoea; the eyes being congested. The next day tremors were noticed all over the body; the eyes were glassy and staring; the mucous membrane of the mouth was cyanotic; the temperature was subnormal, 95°; the blood was dark and contained numerous leucocytes. These symptoms increased; and the animal died in the evening of the following day.

At the post-mortem, numerous subpleural haemorrhages were found; the lungs were normal; the heart pale and soft; the liver was very pale, and showed typical signs of a parenchymatous degeneration; the spleen was enlarged; the kidneys were pale and slightly congested; the medullary and cortical substances not well defined; the mucous membrane of the stomach was congested, and that of the intestines oedematous.

Table III.

Exp. No.	Disease	Date of inoculation	Treatment at the end of—	c.c. of 5 % solution	Relapse after	Remarks
266 A.	*T. gambiense*	Dec. 24	3, 10, 23, 42 days	1·75 c.c.	10 days	Animal living at the end of 93 days.
B.	,,	,,	3 days	0·5 c.c.	—	Lived 4 days. Tryps. absent at death.
C.	,,	,,	3, 10 days	0·75 c.c.	—	Animal still living at the end of 93 days.
267 A.	,,	Dec. 28	3, 13 days	0·75 c.c.	—	Lived 21 days. Spleen small.
B.	,,	,,	,,	0·75 c.c.	—	,, 33 ,, Died of intercurrent diarrhoea.
C.	,,	,,	,,	0·75 c.c.	—	,, 33 ,, ,, ,, ,,
D.	,,	,,	,,	0·75 c.c.	—	,, 21 ,, Spleen small.
278	,,	Jan. 20, 1909	3 days	0·5 c.c.	—	Lived 4 days. Kidneys inflamed. diarrhoea.
360 A.	,,	Jan. 3	4 days	0·5 c.c.	—	Treatment started 6 hrs. before death. Tryps. still present at death, decreased in number.
B.	,,	,,	,,	0·5 c.c.	—	,, ,, ,,
363 A.	,,	Jan. 7	2, 4 days	0·75 c.c.	—	Lived 7 days. Died of abscesses of the lung.
B.	,,	,,	,,	0·75 c.c.	—	,, 6 ,, ,, ,, ,,
372	,,	Jan. 14	5, 14, 22, 38 days	2 c.c.	14, 21, 38	,, 48 days. Tryps. absent at death.
382 A.	,,	Jan. 27	2 days	0·5 c.c.	—	,, 3 ,, ,, ,,
B.	,,	,,	,,	0·5 c.c.	—	,, 6 ,, ,, ,,
271 A.	*T. brucei*	Jan. 4	5, 7, 14 days	1·25 c.c.	—	,, 23 days. Tryps absent at death. Spleen enlarged.
B.	,,	,,	3, 5, 16 days	1·25 c.c.	16 days	,, 31 ,, ,, ,,
C.	,,	,,	3, 5, 16, 28 days	1·75 c.c.	28 days	,, 29 ,, ,, ,,
D.	,,	,,	3, 5 days	1·75 c.c.	—	,, 6 ,, ,, ,,
E.	,,	,,	3, 5, 16 days	1·25 c.c.	—	,, 21 ,, ,, ,, Spleen small.
F.	,,	,,	3, 5, 16 days	1·25 c.c.	—	Animal still living at the end of 86 days.

272 A.	*T. brucei*	Jan. 4	3, 5 days	0·75 c.c.	—	Lived 18 days. Tryps. absent at death.
B.	,,	,,	5 days	0·5 c.c.	—	,, 5 ,,
C.	,,	,,	5 days	0·5 c.c.	—	,, 6 ,, Tryps. absent at death. Spleen enlarged; marked Haemoglobinuria.
D.	,,	,,	3, 5, 7 days	1·0 c.c.	—	,, 12 ,, Tryps. absent at death.
E.	,,	,,	5, 7 days	0·75 c.c.	—	Animal still living at the end of 86 days.
F.	,,	,,	Control	—	—	Died at the end of 5 days.
322 A.	,,	Nov. 28	5, 7 days	1·0 c.c.	—	Lived 7 days. Kidneys inflamed: mucous membrane of intestine oedematous.
B.	,,	,,	,,	1·0 c.c.	—	,, 7 ,, ,, ,, ,, ,,
329 A.	,,	Dec. 3	2 days	0·5 c.c.	—	,, 5 ,, Tryps. absent at death.
B.	,,	,,	,,	0·5 c.c.	—	,, 5 ,, ,, ,,
332 A.	,,	Dec. 5	3 days	0·5 c.c.	—	Animal still living at the end of 115 days.
B.	,,	,,	,,	0·5 c.c.	—	Lived 4 days.
365 A.	,,	Jan. 9	4, 38 days	1·0 c.c.	36 days	Lived 40 days.
B.	,,	,,	3 days	0·3 c.c.	—	,, 3 ,,
325	*T. evansi*	Nov. 30	5, 7 days	0·75 c.c.	15 days	,, 17 days. Died from Trypanosomiasis.
362 A.	,,	Jan. 7	2, 4, 20, 30, 35, 42, 45, 49 days	3·5 c.c.	20, 28, 33, 41-56	,, 56 ,, ,, ,,
367 A.	,,	Jan. 9	3, 4 days	1 c.c.	—	,, 6 ,, Tryps. absent at death.
B.	,,	,,	,,	0·8 c.c.	—	,, 5 ,, ,, ,,

Table IV.

Rat Experiment 362.—

Inoculated with *T. brucei*, January 7.

January 8.	10-15 to field	0·5 c.c. of 5 % *p.* compound
9.	Numerous.	
10.	Neg.	
11.	Neg.	0·25 c.c. „ „ „
26.	Neg.	
27.	1 to 5 fields	0·5 c.c. „ „ „
28.	1-5 to field.	
29.	Neg.	
February 3.	Neg.	
4.	1 to ½ film.	
5.	1 to 20 fields.	
6.	5 to field.	0·5 c.c. „ „ „
7.	Neg.	
8.	Neg.	
9.	1 to ¼ film.	
10.	1 to 2 fields.	
11.	10 to field	0·5 c.c. „ „ „
12.	1 to 20 fields.	
13.	Neg.	
17.	Neg.	
18.	1 to ½ film.	
19.	1 to 10 fields	0·5 c.c. „ „ „
20.	5 to field.	
22.	15 to field	0·5 c.c. „ „ „
23.	15 to field	0·5 c.c. „ „ „
24.	1 to ½ film.	
25.	1 to field.	
26.	5 to field	0·5 c.c. „ „ „
27.		
28.		
March 1.	Increasing in number.	
2.		
3.		
4.	50 to field. *Dead.*	

TABLE V.

Exp. No.	Disease	Weight	Date of inoculation	Treatment at the end of—	c.c. of 10% solution	Relapse after days	Remarks
268	*T. evansi*	10 k. 850 grs.	Jan. 2	16, 19 days	6 c.c., 3 c.c.	—	Lived 23 days. Tryps. absent from peripheral blood at death. Cause of death—Haemorrhagic Nephritis.
269	*T. evansi*	6 k. 100 grs.	Jan. 2	13 days	3 c.c.	—	Lived 15 days. Tryps. present in the blood at death. Cause of death—Haemorrhagic Nephritis.
270	*T. evansi*	6 k. 150 grs.	Jan. 2	13, 16 days	3 c.c., 3 c.c.	—	Lived 20 days. Tryps. absent from peripheral blood at death. Cause of death—Haemorrhagic Nephritis.
280	*T. brucei*	8 k. 500 grs.	Jan. 25	5, 13 days	3 c.c., 6 c.c.	—	Lived 15 days. Tryps. absent from the peripheral blood at death. Cause of death—Haemorrhagic Nephritis.

EXPERIMENT XXVI.—*Macacus rhesus*, weight 2 k. 245 gm. Treatment was begun on the twentieth day after inoculation. The animal was then in a very advanced stage of the disease. The face was puffy, the genitals swollen and oedematous. The blood count gave 1,570,000 red cells, 3,700 white cells and haemoglobin 55 per cent. 0·1 gm. of *p.* compound was then injected. The parasites disappeared about eleven hours after the injection, but the animal was found dead next morning in its cage. The post-mortem revealed the typical lesions of an advanced trypanosomiasis in monkeys.

Two monkeys were inoculated at an earlier stage of the disease.

EXPERIMENT XX.—*Macacus rhesus*, weight 2 k. 540 gm., was injected on the fifteenth day after inoculation with 0·1 gm. of *p.* compound; a relapse set in nine days after the injection, when the same dose was repeated. Thirty-two days afterwards, a third injection of 0·1 gm. of the drug was administered, and then treatment was discontinued. The animal is still alive on the ninety-eighth day after inoculation, and has increased in weight to 2 k. 720 gm. The period of observation is, however, far too short to consider this animal cured.

EXPERIMENT XXIV.—*Macacus rhesus*, weight 1 k. 650 gm. Injected with *T. gambiense*. Very soon the animal showed oedema of the eyelids and oedematous swelling of the genitals. Treatment was begun thirteen days afterwards with an injection of 0·1 gm. of the *p.* compound. The parasites had disappeared from the peripheral circulation by the next day. The same dose was repeated on the twenty-first and thirty-seventh day after inoculation; the treatment was then discontinued. The animal is still alive, sixty-four days after inoculation, and has increased in weight to 1 k. 720 gm.

In order to ascertain the value of a combined Antimony-Atoxyl treatment in monkeys infected with *T. gambiense*, two monkeys (*Macacus rhesus*) were used in the following experiments:—

EXPERIMENT XXII.—*Macacus rhesus*, weight 2 k. 190 gm. The animal was injected twenty-three days after inoculation with 0·1 gm. of *p.* compound. The parasites disappeared promptly from the peripheral circulation. This was followed on the thirty-first day by an injection of 0·1 gm. of Atoxyl. On the forty-eighth day the injection of 0·1 gm. of the *p.* compound was repeated. The animal is still alive, sixty-two days after inoculation, and has regained its original weight.

EXPERIMENT XXV.—*Macacus rhesus*, weight 1 k. 985 gm. It was treated in the same way as in Experiment XXII. This animal is still alive, but in both cases the observation time is far too short to pronounce the animals cured.

CONCLUSIONS

1. The foregoing experiments prove that *p.* and *m.* amino-phenyl-stibinic acids are fairly powerful trypanocides, although their action is not so rapid as that of Sodium-antimonyl-tartrate.

2. That the *p.* amino-phenyl-stibinic acid is decidedly superior in its action to the *m.* amino-phenyl-stibinic acid.

3. Considering the satisfactory results obtained in experimental animals, a trial of the *p.* amino-phenyl-stibinic acid in patients suffering from Sleeping Sickness is justifiable.

4. In our opinion *p.* amino-phenyl-stibinic acid may be administered in the same doses as Atoxyl. As kidney lesions are among the most pronounced results of Antimony poisoning a careful systematic examination of the urine is advisable.

APPENDIX

For the preparation of *p.* and *m.* amino-phenyl-stibinic acid we used the method previously adopted by Michaelis[13] for the production of Di-methyl-amino-phenyl-arsenic acid, employing, however, antimony trichloride instead of arsenic trichloride.

Antimony trichloride was treated with aniline, and the compound $H_2N.C_6H_5.SbCl_2$ obtained, which changed in the presence of alkali into the corresponding hydroxide. This formed, on treatment with hydrogen peroxide in an alkaline solution, aryl-stibinic acid. The process is expressed chemically as follows:—

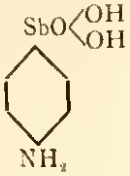
(I) $H_2N \cdot C_6H_5 + SbCl_3 = H_2N \cdot C_6H_4 \cdot SbCl_2 + HCl$

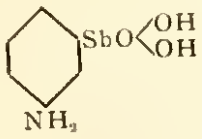
(II) $H_2N \cdot C_6H_4 \cdot SbCl_2 + 2KOH = H_2N \cdot C_6H_4 \cdot Sb(OH)_2$

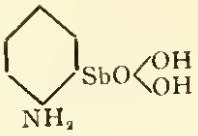
$= H_2N \cdot C_6H_4 \cdot SbO \cdot + H_2O$

(III) $H_2N \cdot C_6H_4 \cdot SbO + H_2O_2 = H_2N \cdot C_6H_4 \cdot SbO(OH)_2$ (Arylstibinic acid)

It is a remarkable fact that on adding aniline to *melting* antimony trichloride, *p.* amino-phenyl-stibinic acid (I) is formed, while, on the other hand, if antimony trichloride is added to *boiling* aniline, *m.* amino-phenyl-stibinic acid (II) is obtained.

I 

II 

III 

For the preparation of *o.* amino-phenyl-stibinic acid (III) we used *Grignar's* reaction, treating *o.* chloraniline with antimonic acid.

Preparation of p. amino-phenyl-stibinic acid.

Thirty grammes of antimony trichloride are heated to 205° in a carefully dried flask for about 6-10 minutes, and 30 grammes of aniline added in three portions. The mixture is kept boiling for 15 minutes and then poured into 500 c.c. of water. The precipitate which is formed is collected on a filter paper and then added to a hot solution of sodium carbonate, which dissolves free antimonic acid. It is then boiled with 80 c.c. of strong potassium hydrate (25%) for three

hours, diluted, and 75 c.c. of commercial hydrogen peroxide added. At the end of two days the precipitate is boiled for two hours and filtered. On acidifying with diluted sulphuric acid; the *p.* amino-phenyl-stibinic acid is then obtained, which crystallizes in small needles from alcohol and water (1 : 3) and readily forms a sodium salt. The latter does not melt under 360° C., but turns brown at 323° C.-326° C. When treated with potassium iodide and sulphuric acid it is easily converted into *p.* iodo-aniline (M.P. 61° C.).

Below is an analysis of the sodium salt.

$(C_6H_4O_3NSbNa)$

	Calculated	Found	(2)
C	25·26 per cent.	24·37 per cent.	25·17 per cent.
H	2·80 ,,	3·04 ,,	2·92 ,,
N	4·91 ,,	5·18 ,,	5·04 ,,
Sb	42·11 ,,	42·63 ,,	42·43 ,,
Na	8·06 ,,	8·19 ,,	8·27 ,,

Preparation of m. amino-phenyl-stibinic acid.

Twelve grammes of aniline are kept boiling, using an air condenser, and fifteen grammes of antimony trichloride are added in small portions. After all the antimony trichloride is dissolved, the temperature is kept up to 165° for three hours, and then the mixture poured into 500 c.cm. of water. The further procedure is the same as for the preparation of *p.* amino-phenyl-stibinic acid. The acid crystallizes from alcohol in long needles and melts at 207° C.-208° C When treated with potassium iodide and sulphuric acid it forms, though very sparingly, *m.* iodo-aniline (M.P. 26° C.).

It is easily acetylated on boiling with acetic anhydride for two hours, and yields small needles which crystallize from diluted alcohol (1 : 15). M.P. 186° C.-188° C.

Below is an analysis of the sodium salt of *m.* amino-phenyl-stibinic acid : —

$(C_6H_4O_3NSbNa)$

	Calculated	Found (1)	(2)
C	25·26 per cent.	25·03 per cent.	—
H	2 80 ,,	3·12 ,,	—
N	4·91 ,,	4·72 ,,	—
Sb	42·11 ,,	42·76 ,,	42·26 per cent.
Na	8·06 ,,	8·14 ,,	8·19 ,,

Preparation of o. amino-phenyl-stibinic acid.

Two grammes of *o.* chlor-aniline are dissolved in 30 c.c. of *dry* ether, and one gramme of antimonic acid added. After carefully drying for two hours at 200° C., 0·4 gramme of magnesium is added. The temperature rises to 45° C., and is kept at this temperature for 1-1½ hours. After evaporation of the ether, the residue is shaken up with 25 c.c. of sodium carbonate solution (5%), and the mixture is then warmed on a steam bath for one hour and filtered. The filtrate is acidified with diluted sulphuric acid, and the resulting precipitate dried and extracted with absolute alcohol. On evaporation of the alcohol, *o.* amino-phenyl-stibinic acid is left, which crystallizes from a mixture of alcohol and pyridine (1 : 3) in leaflets. The *o.* amino-phenyl-stibinic acid melts at 192° C. to 194° C., and forms an acetyl derivative on boiling with acetic anhydride (M.P. 167° C.-169°C.). On treatment with potassium iodide and sulphuric acid it yields *o.* iodo-aniline (M.P. 54° C.).

$(C_6H_4O_3NSbNa)$

	Calculated		Found	
C	25·26	per cent.	25·12	per cent.
H	2·80	,,	2·93	,,
N	4·91	,,	5·16	,,
Sb	42·11	,,	42·76	,,
Na	8·06	,,	8·23	,,

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